ELSEVIER

Biochimica et Biophysica Acta 1587 (2002) 99–106

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.bba-direct.com

Overexpression of kidney phosphatidylinositol 4-kinaseβ and phospholipase $C_{\gamma 1}$ proteins in two rodent models of polycystic kidney disease☆

F.P. Cuozzo [a,b], S. Mishra [a,b], J. Jiang [c], H.M. Aukema [a,b,c,d,*]

[a] *Department of Biology, Texas Woman's University, Denton TX, 76204, USA*
[b] *Department of Nutrition and Food Sciences, Texas Woman's University, Denton TX, 76204, USA*
[c] *Department of Human Nutritional Sciences, University of Manitoba, R3T 2N2 Winnipeg, Canada*
[d] *Department of Pediatrics and Child Health, University of Manitoba, R3T 2N2 Winnipeg, Canada*

Received 17 December 2001; received in revised form 14 March 2002; accepted 14 March 2002

Abstract

Our studies of renal phosphoinositide levels and metabolism in the *pcy* mouse with polycystic kidney disease (PKD) suggest that phosphatidylinositol kinase (PtdInsK) and phospholipase C (PLC) are elevated in this renal disorder. Therefore, the steady-state levels of select isoforms of these enzymes were examined in renal cytosolic and particulate (detergent-soluble) fractions in male and female normal and CD1-*pcy*/*pcy* (*pcy*) mice at 60, 120 and 180 days of age, and in male and female normal and diseased (Han:SPRD-*cy*) rats at 28 and 70 days of age. Disease-related increases in phosphatidylinositol 4-kinaseβ (PtdIns4Kβ) and $PLC_{\gamma 1}$ levels were present in both models. PtdIns4Kβ levels were higher by as much as 233% in *pcy* mice and by 95% in diseased Han:SPRD-*cy* rats compared to normals of the same age and gender. Steady-state levels of $PLC_{\gamma 1}$ were as much as 74% and 35% higher in *pcy* mice and diseased Han:SPRD-*cy* rats, respectively, compared to their controls. The consistency of these alterations in two accepted models of PKD indicates the importance of the phosphoinositide signalling pathway in the evolution of this disorder, and represents a potential site for therapeutic intervention.

Keywords: Phosphatidylinositol 4-kinaseβ; Phospholipase $C_{\gamma 1}$; Phosphoinositide metabolism; Polycystic kidney disease; Immunoblotting; Animal model

1. Introduction

Polycystic kidney disease (PKD) encompasses a variety of kidney cystic disorders that are characterized by abnormal renal cyst growth and development. The autosomal dominant form is a hereditary disorder that affects approximately 1 in 1000 people, and causes renal death at an average age of 50–55 years [1]. Polycystic kidneys contain hundreds of fluid filled cysts which cause increased renal size in humans and ultimately renal failure.

While the presence of PKD is genetically determined, the rate of disease progression is significantly influenced by mediators of the cell environment, such as growth factors and dietary factors [2]. Growth factor abnormalities include epidermal growth factor (EGF), transforming growth factor-α and -β (TGFα and TGFβ), hepatocyte growth factor and insulin-like growth factor-I (IGF-I). Activation of the receptors for many of the growth factors that are altered in PKD results in autophosphorylation at tyrosine residues and phosphorylation of other substrates, including SH_2-containing proteins.

In relation to this, we demonstrated that phosphoinositide metabolism, which is regulated in part by tyrosine phosphorylation, is altered in the *pcy* model of PKD [3–6]. Using the DBA-*pcy/pcy* mouse, mass analysis revealed that the level of phosphatidylinositol (PtdIns) is considerably lower in *pcy* mouse kidneys, while the level of PtdIns-phosphate (PtdInsP) is not different [3,6]. As a result, the PtdInsP/PtdIns ratio is higher in *pcy* mouse kidneys. In a tracer isotope study [4], *pcy* and normal mice were injected intraperitoneally with [^{3}H]inositol and sacrificed 12 min later, so that the radioactivity in the phosphoinositides predominantly reflects synthesis. The [^{3}H]PtdInsP/[^{3}H]PtdIns ratio also was elevated in this study, suggesting that the

☆ Portions of this work were presented in abstract form [FASEB J. 13 (1999) A1096].

* Corresponding author. Department of Human Nutritional Sciences, University of Manitoba, R3T 2N2 Winnipeg, Manitoba, Canada. Tel.: +1-204-474-8076; fax: 1+204-474-7593.
E-mail address: Aukema@UManitoba.CA (H.M. Aukema).

0925-4439/02/$ - see front matter
PII: S0925-4439(02)00072-8

conversion of PtdIns to PtdInsP was elevated in *pcy* mouse kidneys.

In addition, both mass and radiolabelled PtdIns-4,5-bisphosphate (PtdIns-4,5-P_2) as well as the PtdIns-4,5-P_2/PtdInsP ratio are lower in *pcy* mouse kidneys compared to normals [4]. This lower level of renal PtdIns-4,5-P_2 in *pcy* compared to normal mice could be due to decreased synthesis or increased degradation of this phosphoinositide. Our mass and labelling studies indicate that synthesis of PtdIns-4,5-P_2 (via PtdInsP 5-kinase activity) is not different in *pcy* compared to normal mouse kidneys, but that degradation of this phosphoinositide may be elevated in the diseased kidneys. In addition, renal diacylglycerol levels are elevated in *pcy* mice [5], consistent with increased level and/or activity of a phosphoinositide-specific phospholipase C (PLC) in *pcy* mouse kidneys compared with normals.

Since several growth factors are altered in PKD, specific isoforms of PLC and PtdIns kinase (PtdInsK) that are activated by tyrosine phosphorylation following growth factor receptor activation [7–11], were examined by immunoblot analysis. To test our hypothesis that the levels of phosphatidylinositol 3-kinase (PtdIns3K), PtdIns4Kβ and $PLC_{\gamma 1}$ were higher in PKD kidneys, we first used the CD1-*pcy/pcy* (*pcy*) mouse model of PKD in which the decline in renal function begins in adulthood. We confirmed that these findings are not an anomaly of this model by using another model of PKD in which the decline in renal function occurs during the growth phase, namely the Han:SPRD-*cy* rat.

2. Methods

2.1. Animals

The CD1-*pcy/pcy* (*pcy*) mouse and the Han:SPRD-*cy* rat used in this study are well-characterized and accepted models of autosomal dominant PKD [12,13]. The animal experimental protocol was in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals and was approved by the University Animal Care and Use Committees. Animals were from our colonies, which were established from breeding stock obtained from V.H. Gattone II (*pcy* mice) and B.D. Cowley (Han:SPRD-*cy* rats), University of Kansas Medical Centre. All animals were housed in temperature- (22 to 24 °C), humidity- (50% to 60% relative humidity) and light- (12-h light/dark cycle) controlled conditions. Animals were weaned between 3 and 4 weeks of age and received standard lab chow and tap water, which were provided ad libitum. Normal and diseased mice ($n=5-6$) were killed at 60, 120 or 180 days and normal and diseased rats ($n=6-10$) were killed at 28 or 70 days. For the mice, cyst development is slow and decline in markers of renal function does not usually occur until after 6 months of age [13,14]. In contrast, cyst growth is rapid in the rat and markers of renal function are significantly altered by 70 days of age [12,15]. Animals were lightly anesthetized with CO_2 before decapitation and tissues were removed, weighed and immediately frozen in liquid nitrogen.

2.2. Sample preparation

Tissues were lyophilized and a representative sample was homogenized in 100 volumes of ice-cold homogenization buffer [50 mM Tris–HCl [pH 7.4], 250 mM sucrose, 2 mM EDTA, 1 mM EGTA, 10 mM β-mercaptoethanol, 100 μM sodium orthovanadate, 1 μg/ml soybean trypsin inhibitor, 144 μM 4-(2-aminoethyl) benzene-sulfonyl fluoride, and 25 μg/ml of aprotinin, pepstatin and leupeptin (all reagents from Sigma, St. Louis, MO)] [16]. Homogenates were centrifuged at 100,000 × *g* for 30 min at 4 °C and the supernatant, which represents the cytosolic fraction, was collected. The remaining pellet was resuspended in 15

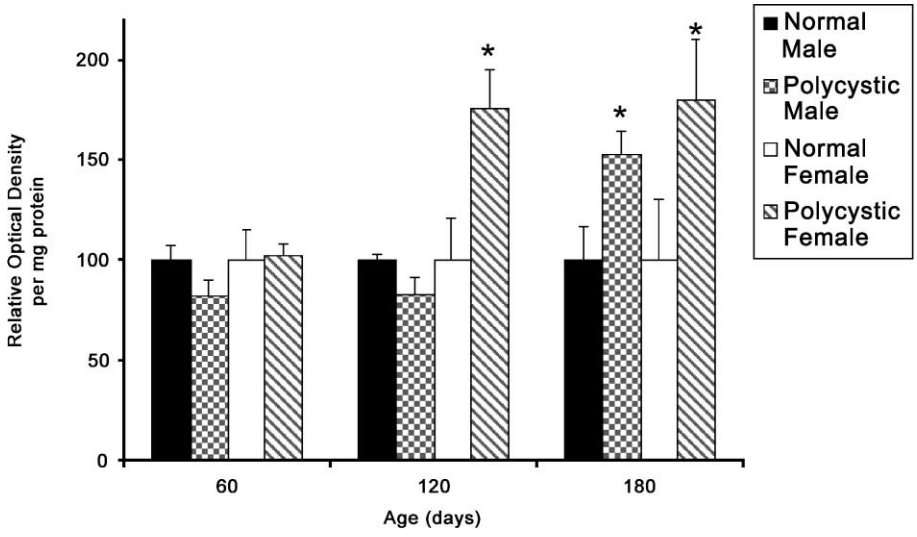

Fig. 1. Steady state levels of PtdIns4Kβ in normal and *pcy* mouse kidney cytosolic fractions. * $P<0.05$, significantly different from same gender and age normals.

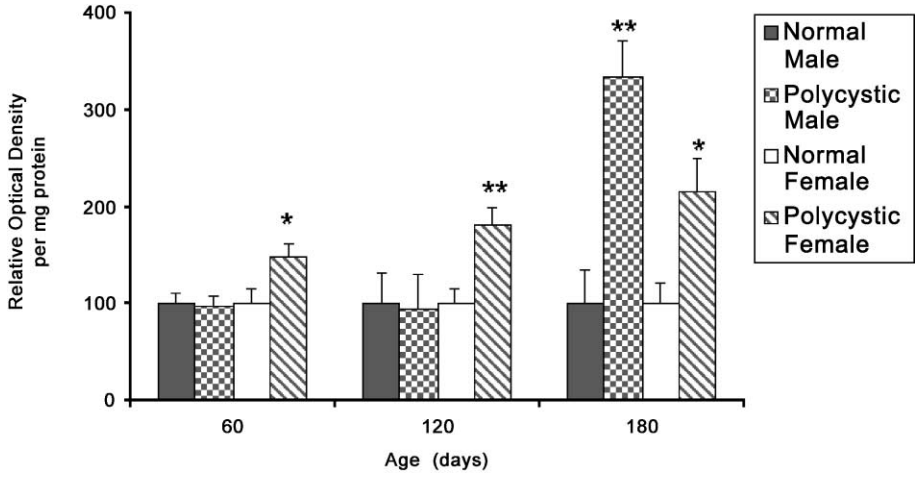

Fig. 2. Steady state levels of PtdIns4Kβ in normal and *pcy* mouse kidney particulate fractions. * $P<0.05$, ** $P<0.01$, significantly different from same gender and age normals.

volumes of the homogenization buffer containing 1% Triton X-100 (Sigma), incubated on ice for 10 min and centrifuged at 100,000 × *g* for 30 min at 4 °C. The resulting supernatant was collected as the particulate extract and represents the Triton-soluble fractions of the plasma and intracellular membranes. Homogenates were stored at −80 °C until analyses. Protein concentrations of all fractions were determined by protein assay as described by Bradford [17], with bovine serum albumin as the standard.

2.3. Immunoblotting

Samples were prepared for immunoblotting using Laemmli sample buffer [18], and electrophoresed for 2 h at 200 V on 7.5% SDS-PAGE minigels with 4% stacking gels. Following electrophoretic protein separation, the gels were soaked in transfer buffer for 10 min and the proteins were transferred onto PVDF membranes at 375 mA for a minimum of 2 h. After removal from the transfer apparatus, the membranes were blocked with 5% skim milk or goat serum (Jackson ImmunoResearch Laboratories Inc., USA) in Tris-buffered saline with 0.1% Tween. Primary antibodies to PtdIns3K, PtdIns4Kβ and $PLC_{\gamma 1}$ (catalogue numbers 06-195, 06-578 and 06152, respectively) were obtained from Upstate Biotechnology (USA). Incubation time was at least 12 h at 4 °C. For mouse tissues, antibody titres for PtdIns3K, PtdIns4Kβ and $PLC_{\gamma 1}$ were 1:2000, 1:10,000 and 1:500, respectively. For rat kidneys, dilutions of 1:2000 were used for PtdIns4Kβ and $PLC_{\gamma 1}$.

For the mouse tissues, all secondary antibody incubations were 2 h at room temperature in a 1:10,000 dilution of an alkaline phosphatase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories). Bands were visualized by developing the membranes with NBT/BCIP (Gibco BRL). The MCID system (Imaging Research, St. Catharines, ONT, Canada) was employed to determine the optical densities of the bands of interest. For the rat tissues, a peroxidase-conjugated secondary antibody was incubated for 1 h at room temperature at a dilution of 1:50,000. Membranes were then incubated with Chemi Glow™ (Alpha Innotech, San Leandro, CA) and image analysis and quantitation of immunoreactive bands was performed using the Fluorchem Imager (Alpha Innotech).

A range of protein amounts was loaded onto gels for each antibody to ensure that the quantitative comparisons could be made for results within each isoform. The amount of protein that was in the middle of the linear response and used for analyses of the enzymes of interest ranged from 1 to 14 μg of protein. Colour molecular weight markers, Jurkat cell lysates and bovine brain cytosol were used as standards to verify the identity of the bands of interest. Negative controls included using a nonspecific antibody (anti-ovalbumin) or omitting the primary or secondary antibody.

2.4. Statistical analyses

All samples from one age and gender group were loaded on the same gel with the same amount of protein and were

Table 1
Steady-state levels (O.D./mg protein) of PtdIns4Kβ and $PLC_{\gamma 1}$ in livers of *pcy* mice

Age (days)	Gender	PtdIns4Kβ		$PLC_{\gamma 1}$	
		Cytosolic	Particulate	Cytosolic	Particulate
60	male	117 ± 11	120 ± 33	67 ± 13	90 ± 16
120	male	108 ± 5	107 ± 12	86 ± 14	84 ± 6
180	male	126 ± 11	83 ± 13	85 ± 5	77 ± 11
60	female	111 ± 11	79 ± 19	68 ± 14	98 ± 13
120	female	89 ± 13	116 ± 27	103 ± 8	142 ± 25
180	female	88 ± 5	70 ± 8*	80 ± 3*	63 ± 9*

Data are mean optical density ± S.E., expressed relative to normal controls of the same age and gender. PtdIns4Kβ, phosphatidylinositol 4-kinaseβ; $PLC_{\gamma 1}$, phospholipase $PLC_{\gamma 1}$.

* $P<0.05$, compared to normal control of same age and gender.

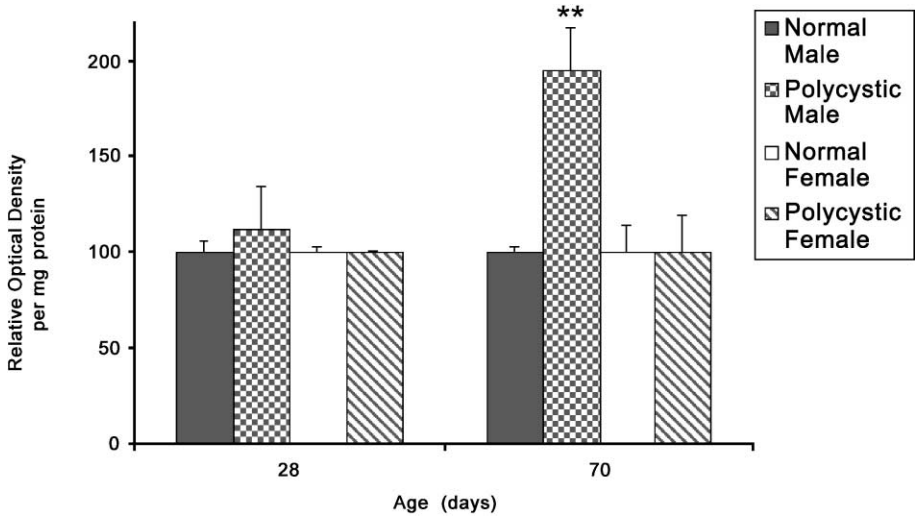

Fig. 3. Steady state levels of PtdIns4Kβ in normal and polycystic Han:SPRD-*cy* rat kidney particulate fractions. ** $P < 0.01$, significantly different from same gender and age normals.

analyzed together. Data was analyzed by Student's *t*-test when the data displayed homogeneity of variance. If variances were not equal, as detected by either Levene's or Bartlett's test, significant differences were determined using Wilcoxon's test. Data are expressed as mean ± S.E. for the normal and diseased groups, with the mean of the normal controls equated to 100 and the other values expressed relative to the normal group.

3. Results

3.1. PtdInsK levels

Immunoblot analysis demonstrated the presence of PtdIns4Kβ in the kidneys and livers of *pcy* and control mice in both the cytosolic and particulate fractions. In the kidneys of 180-day-old *pcy* mice compared to normals, the level of PtdIns4Kβ in the cytosolic and particulate fractions was elevated in both males (by 53% and 233%, respectively) and females (by 80% and 115%, respectively) (Figs. 1 and 2). In females, the steady-state levels of PtdIns4Kβ were higher by approximately 48% in the particulate fraction of 60-day-old mice, and by 80% in both fractions at 120 days of age. In contrast to the diseased kidneys, the levels of PtdIns4Kβ in livers from *pcy* mice with PKD were not elevated compared to controls, with the only difference being a 30% lower level of PtdIns4Kβ in the particulate fraction of livers from 180-day-old female *pcy* mice compared to controls (Table 1).

To determine whether the renal alterations were present in another animal model of PKD, the kidneys of Han:SPRD-*cy* rats were analyzed for PtdIns4Kβ. In both models, the progression of cyst growth in PKD is reflected in changes in

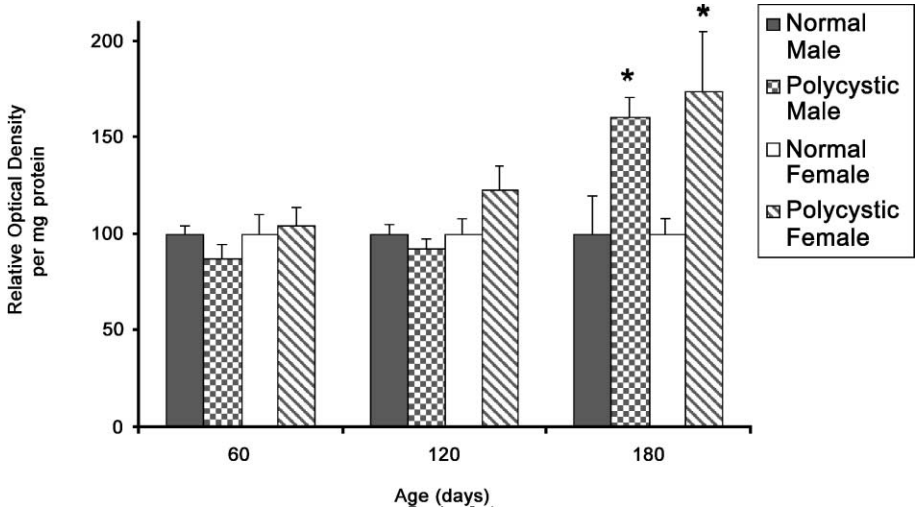

Fig. 4. Steady state levels of $PLC_{\gamma 1}$ in normal and *pcy* mouse kidney cytosolic fractions. * $P < 0.05$, significantly different from same gender and age normals.

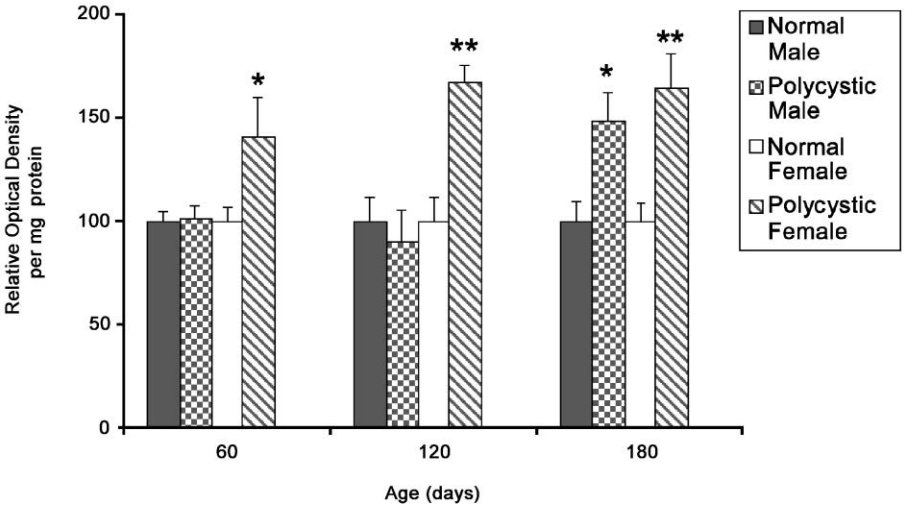

Fig. 5. Steady state levels of $PLC_{\gamma 1}$ in normal and *pcy* mouse kidney particulate fractions. * $P<0.05$, ** $P<0.01$, significantly different from same gender and age normals.

renal size in diseased animals compared to normals. In 180-day-old mice, kidney weight relative to body weight (g/100 g) in *pcy* compared to normal animals was 1.60 ± 0.12 vs. 0.79 ± 0.08 ($P<0.001$) in males and 1.90 ± 0.22 vs. 0.58 ± 0.03 ($P<0.001$) in females. A more than doubling of kidney weight also occurred by 70 days in male Han:SPRD-*cy* rats with relative kidney weights of 1.06 ± 0.07 vs. 0.44 ± 0.01 ($P<0.001$) in diseased compared to normal animals. In contrast, the disease is less aggressive in female rats, with relative kidney weights of 0.58 ± 0.02 vs. 0.38 ± 0.01 ($P<0.001$) in diseased compared to normal animals. Significant differences in PtdIns4Kβ levels were observed in only the particulate fraction from renal extracts of 70-day-old male Han:SPRD-*cy* rats, where PtdIns4Kβ levels were 95% higher in diseased compared to normal rats (Fig. 3).

Our previous data using in vivo labelling of phosphoinositides in *pcy* mice demonstrated that more than 98% of the PtdInsP activity was associated with the PtdIns-4-P isomer, but that small amounts of PtdIns-3-P also were present. Immunoblot analyses of *pcy* mouse kidneys and livers, however, indicate that the levels of PtdIns3K are not altered (data not shown).

3.2. $PLC_{\gamma 1}$ levels

The pattern of $PLC_{\gamma 1}$ expression in the kidneys and livers of *pcy* mice was similar to that of PtdIns4Kβ. In 180-day-old *pcy* mice compared to normals, renal levels of $PLC_{\gamma 1}$ were higher in cytosolic and particulate fractions in both male (by 61% and 48%, respectively) and female (by 74% and 64%, respectively) animals (Figs. 4 and 5). In 60-

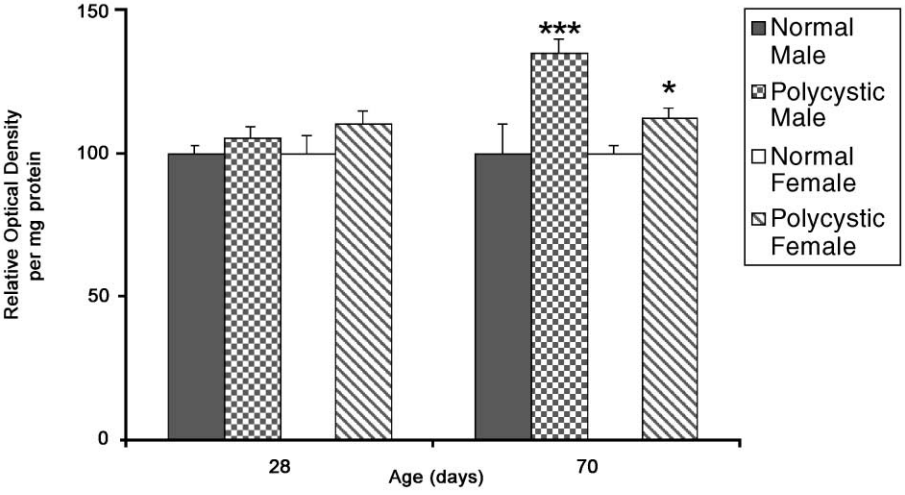

Fig. 6. Steady state levels of $PLC_{\gamma 1}$ in normal and polycystic Han:SPRD-*cy* rat kidney cytosolic fractions. * $P<0.05$, *** $P<0.001$, significantly different from same gender and age normals.

and 120-day-old *pcy* females, $PLC_{\gamma 1}$ was 41% and 67% higher, respectively, in the particulate fractions of the kidneys. $PLC_{\gamma 1}$ levels were not elevated in the livers of *pcy* mice, with the only significant differences being in the cytosolic and particulate fractions of livers from 180-day-old female animals (20% and 37% lower, respectively) (Table 1). In the Han:SPRD-*cy* rats, $PLC_{\gamma 1}$ levels were elevated in the cytosolic fractions of kidneys from 70-day-old males (by 35%) and females (by 12%) (Fig. 6). $PLC_{\gamma 1}$ levels in particulate fractions did not appear to differ between extracts from normal and diseased kidneys. However, the low levels present precluded accurate quantification of these bands.

4. Discussion

The current study demonstrates that the levels of specific enzymes PtdIns4Kβ and $PLC_{\gamma 1}$ are elevated not only in *pcy* mouse kidneys, but also in the polycystic kidneys of another accepted animal model of PKD, namely the Han:SPRD-*cy* rat. This is consistent with our previous reports on phosphoinositide metabolism in the *pcy* mouse in which mass quantitation and tracer radioisotope analyses suggested that the activity of renal PtdInsKs and PLC are elevated in this model of PKD [3–6].

Both PtdIns4Kβ and $PLC_{\gamma 1}$ are downstream of growth factor receptors that are activated via tyrosine phosphorylation events [8,10,19–21]. The level of several growth factors and their receptors are known to be upregulated in PKD and in animal models of this disease. EGF concentrations are altered in PKD [22–25], and hyperresponsiveness to EGF has been demonstrated in cultured cells derived from PKD cystic epithelium [25]. The distribution and metabolism of EGF is abnormal in several mouse models of PKD [24,26–28], and the EGF receptor is mislocated to the apical membrane of renal epithelial cells derived from mice with PKD and from PKD patients [1]. EGF and IGF-I also are found in renal cyst fluid [27,29,30]. Both EGF receptor and TGFα are overexpressed in human PKD [31]. The expression of the TGFα transgene in the kidney results in the formation of renal cysts in otherwise normal mice and in the acceleration of cyst enlargement in *pcy* mice with PKD [26,32]. TGFα is a close relative of EGF, and like EGF, exerts its effect on cells through binding to the EGF receptor. In addition to EGF, gene expression of IGF-I, platelet-derived growth factor and fibroblast growth factor also is affected in the *pcy* animal model of PKD [28]. In the Han:SPRD-*cy* rat, serum and kidney levels of IGF-I are elevated in diseased animals compared to normals [15].

Increased tyrosine kinase activity of the EGF receptor in cystic renal tissue is a functional event that is part of the disease pathway of renal cyst formation. This has been demonstrated in an in vitro model of tubular cyst development in which inhibition of tyrosine kinase activity resulted in a reduction in tubular epithelial cell hyperplasia [33]. Furthermore, in vivo inhibition of the EGF receptor or inhibiting EGF tyrosine kinase activity in an animal model of PKD slows disease progression [34–36], indicating the importance of tyrosine kinase-mediated events in PKD. EGF stimulates the activity of $PLC_{\gamma 1}$ and PtdIns4Kβ, and coprecipitation studies demonstrate that the EGF receptor is associated with PtdIns4Kβ and $PLC_{\gamma 1}$ [19,21,37–39]. Activation of these enzymes resulting in phosphoinositide turnover results in the generation of the intracellular signalling molecules, diacylglycerol and inositol trisphosphate. These second messengers initiate many activities associated with increased cell proliferation and growth, consistent with the increased epithelial cell proliferation observed in PKD [40,41].

Binding of growth factors to their receptors results in autophosphorylation of the receptor itself and other proteins. This autophosphorylation creates high affinity binding sites for SH2 domain-containing proteins including the receptor, resulting in its dimerization and recruitment of SH2 domain-containing proteins such as $PLC_{\gamma 1}$. Hence, upon stimulation, $PLC_{\gamma 1}$ translocates from the cytosol to the membrane of the cell, where it is activated and acts on its substrate, PtdIns-4,5-P_2 [42,43]. With activation, therefore, one would expect increased levels of $PLC_{\gamma 1}$ associated with the membrane fraction and a concomitant decrease in this protein in the cytosol. In our studies, however, we observed an increase in the levels of $PLC_{\gamma 1}$ in both the cytosolic and membrane-associated fractions, suggesting an overall increase in the expression of this protein. Overexpression of $PLC_{\gamma 1}$ protein occurs in various carcinomas including human breast and colorectal cancer tissues [44,45] and in hyperproliferative epidermal diseases [46]. The regulation of $PLC_{\gamma 1}$ expression has not been well characterized, but the activation of the vitamin D-responsive element has been shown to increase $PLC_{\gamma 1}$ expression [47]. Although little is known also regarding the control of PtdIns4K expression, elevated PtdIns4K activity is associated with cell proliferation in tumours, newborn and regenerating liver, thymus, bone marrow, spleen and testis [48]. Our study demonstrates that the levels of PtdIns4K and $PLC_{\gamma 1}$ are elevated in PKD kidneys of two animal models. However, the mechanism by which the expression of these proteins is altered in diseased kidneys, whether increased expression is found in other hyperproliferative renal disorders and how this is regulated remains to be determined.

Although PtdIns-4-P is the predominant isomer of PtdInsP in the kidney, low levels of PtdIns-3-P are also found in the kidney, with the level of isotopically labelled PtdIns-3-P being less than 2% of total PtdInsP [3,4]. In one study, the relative amount of PtdIns-3-P was not different in normal compared to *pcy* mouse kidneys, although the level of this isomer did correlate with increasing kidney size in cystic kidneys [4]. In the current study, the levels of PtdIns3K were not different in *pcy* mouse kidneys compared to controls, consistent with the findings on the levels of this

specific phosphoinositide isomer. The fact that the level of PtdIns3K was not elevated suggests that the higher PtdIns4Kβ and $PLC_{\gamma 1}$ levels are not simply part of a generalized increase in tyrosine kinase activity and phosphoinositide turnover, but represent specific alterations in these diseased kidneys which remain to be elucidated.

Our previous studies indicated that hepatic phosphoinositide levels were not altered in the *pcy* mice [3]. In contrast to human PKD, in which hepatic cysts commonly occur, the livers in these mice do not contain cysts, and the levels of PtdIns4Kβ and $PLC_{\gamma 1}$ were not elevated in this tissue, suggesting that the phosphoinositide alterations seen in the kidney are specific for the diseased tissue. Our data do not explain why the levels of these enzymes are lower in the liver fractions of 180-day-old *pcy* mice. However, reduced levels of these enzymes is consistent with the reduced growth of the liver and lower body weights in these animals [3].

The greater changes in enzyme alterations in male Han:SPRD-*cy* rats is likely a reflection of the greater severity of disease in males in this model of PKD. The reason for the earlier appearance of these abnormalities in the female compared to male *pcy* mouse, however, is not readily apparent. Initial reports of this model with the *pcy* mutation on the DBA/2J background reported that cystic females developed severe uremia and died sooner than their male counterparts. However, we have observed that disease progression in male and female *pcy* mice with the *pcy* mutation on the CD1 background occurs at similar rates, and on some diets disease progression is slower in females than in males [14,49].

The overexpression of these enzymes in two distinct models of PKD demonstrates the importance of the phosphoinositide signalling pathway in this disorder. It is clear that inhibition of EGF signalling can reduce disease progression [34–36]. Strategies which alter enzymes in growth factor-mediated phosphoinositide signalling pathways may offer opportunities for therapeutic interventions in this disorder and further our understanding of molecular events which regulate the expression of these proteins.

Acknowledgements

This work was supported by research grants from the National Institutes of Health and the Natural Sciences and Engineering Research Council of Canada.

References

[1] P.D. Wilson, D. Falkenstein, Curr. Top. Pathol. 88 (1995) 1–50.
[2] Q. Qian, P.C. Harris, V.E. Torres, Kidney Int. 59 (2001) 2005–2022.
[3] H.M. Aukema, T. Yamaguchi, K. Tomobe, D.J. Philbrick, R.S. Chapkin, H. Takahashi, B.J. Holub, J. Nutr. 125 (1995) 1183–1191.
[4] H.M. Aukema, R.S. Chapkin, K. Tomobe, H. Takahashi, B.J. Holub, Exp. Mol. Pathol. 57 (1992) 39–46.
[5] H.M. Aukema, K. Tomobe, H. Takahashi, B.J. Holub, J. Nutr. Biochem. 3 (1992) 491–495.
[6] H.M. Aukema, T. Yamaguchi, H. Takahashi, B. Celi, B.J. Holub, Lipids 27 (1992) 429–435.
[7] M.P. Wymann, L. Pirola, Biochim. Biophys Acta 1436 (1998) 127–150.
[8] S.G. Rhee, Y.S. Bae, J. Biol. Chem. 272 (1997) 15045–15048.
[9] D.A. Fruman, R.E. Meyers, L.C. Cantley, Annu. Rev. Biochem. 67 (1998) 481–507.
[10] M. Katan, Biochim. Biophys. Acta 1436 (1998) 5–17.
[11] T. Balla, Biochim. Biophys. Acta 1436 (1998) 69–85.
[12] B.D. Cowley Jr., S. Gudapaty, A.L. Kraybill, B.D. Barash, M.A. Harding, J.P. Calvet, V.H. Gattone II, Kidney Int. 43 (1993) 522–534.
[13] H. Takahashi, J.P. Calvet, D. Dittemore-Hoover, K. Yoshida, J.J. Grantham, V.H. Gattone II, J. Am. Soc. Nephrol. 1 (1991) 980–989.
[14] H.M. Aukema, I. Housini, J.M. Rawling, J. Am. Soc. Nephrol. 10 (1999) 300–308.
[15] H.M. Aukema, I. Housini, Kidney Int. 59 (2001) 52–61.
[16] H.M. Aukema, L.A. Davidson, W.C. Chang, J.R. Lupton, J.N. Derr, R.S. Chapkin, Biochim. Biophys. Acta 1224 (1994) 51–60.
[17] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[18] U.K. Laemmli, Nature 227 (1970) 680–685.
[19] B. Margolis, F. Bellot, A.M. Honegger, A. Ullrich, J. Schlessinger, A. Zilberstein, Mol. Cell. Biol. 10 (1990) 435–441.
[20] D.H. Walker, L.J. Pike, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 7513–7517.
[21] C. Cochet, O. Filhol, B. Payrastre, T. Hunter, G.N. Gill, J. Biol. Chem. 266 (1991) 637–644.
[22] J.P. Calvet, Kidney Int. 43 (1993) 101–108.
[23] J. Lakshmanan, V. Eysselein, Biochem. Biophys. Res. Commun. 197 (1993) 1083–1093.
[24] S.A. Orellana, W.E. Sweeney, C.D. Neff, E.D. Avner, Kidney Int. 47 (1995) 490–499.
[25] P.D. Wilson, A.C. Sherwood, Kidney Int. 39 (1991) 450–463.
[26] V.H. Gattone II, K.A. Kuenstler, G.W. Lindemann, X. Lu , B.D. Cowley Jr., C.A. Rankin, J.P. Calvet, J. Lab. Clin. Med. 127 (1996) 214–222.
[27] S. Horikoshi, S. Kubota, G.R. Martin, Y. Yamada, P.E. Klotman, Kidney Int. 39 (1991) 57–62.
[28] T. Nakamura, I. Ebihara, I. Nagaoka, Y. Tomino, S. Nagao, H. Takahashi, H. Koide, J. Am. Soc. Nephrol. 3 (1993) 1378–1386.
[29] D.W. Moskowitz, S.L. Bonar, W. Liu, C.F. Sirgi, M.D. Marcus, R.V. Clayman, J. Urol. 153 (1995) 578–583.
[30] M. Taide, S. Kanda, T. Igawa, J. Eguchi, H. Kanetake, Y. Saito, Eur. J. Clin. Invest. 26 (1996) 506–513.
[31] D.C. Lee, K.W. Chan, S.Y. Chan, J. Urol. 159 (1998) 291–296.
[32] D.A. Lowden, G.W. Lindemann, G. Merlino, B.D. Barash, J.P. Calvet , V.H. Gattone II, J. Lab. Clin. Med. 124 (1994) 386–394.
[33] E.D. Avner , W.E. Sweeney Jr., D. Ellis, Kidney Int. 36 (1989) 960–968.
[34] W.G. Richards, W.E. Sweeney, B.K. Yoder, J.E. Wilkinson, R.P. Woychik, E.D. Avner, J. Clin. Invest. 101 (1998) 935–939.
[35] W.E. Sweeney, Y. Chen, K. Nakanishi, P. Frost, E.D. Avner, Kidney Int. 57 (2000) 33–40.
[36] F. Terzi, M. Burtin, M. Hekmati, P. Federici, G. Grimber, P. Briand, G. Friedlander, J. Clin. Invest. 106 (2000) 225–234.
[37] A. Kauffmann-Zeh, G.M. Thomas, A. Ball, S. Prosser, E. Cunningham, S. Cockcroft, J.J. Hsuan, Science 268 (1995) 1188–1190.
[38] R. Meyers, L.C. Cantley, J. Biol. Chem. 272 (1997) 4384–4390.
[39] L.J. Pike, A.T. Eakes, J. Biol. Chem. 262 (1987) 1644–1651.
[40] J.P. Calvet, J.J. Grantham, Semin. Nephrol. 21 (2001) 107–123.
[41] C. Marfella-Scivittaro, A. Quinones, S.A. Orellana, Am. J. Physiol., Cell Physiol. 282 (2002) C693–C707.
[42] A.M. Soderquist, G. Todderud, G. Carpenter, Cancer Res. 52 (1992) 4526–4529.

[43] G. Todderud, M.I. Wahl, S.G. Rhee, G. Carpenter, Science 249 (1990) 296–298.
[44] D.Y. Noh, Y.H. Lee, S.S. Kim, Y.I. Kim, S.H. Ryu, P.G. Suh, J.G. Park, Cancer 73 (1994) 36–41.
[45] C.L. Arteaga, M.D. Johnson, G. Todderud, R.J. Coffey, G. Carpenter, D.L. Page, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 10435–10439.
[46] L.B. Nanney, R.E. Gates, G. Todderud, L.E. King Jr., G. Carpenter, Cell Growth Differ. 3 (1992) 233–239.
[47] Z. Xie, D.D. Bikle, J. Biol. Chem. 272 (1997) 6573–6577.
[48] M.T. Rizzo, G. Weber, Cancer Res. 54 (1994) 2611–2614.
[49] H.M. Aukema, T. Yamaguchi, H. Takahashi, D.J. Philbrick, B.J. Holub, Nutr. Res. 12 (1992) 1383–1392.